

CLAIM LISTING

The listing of claims will replace all prior listings of claims in the application:

Claims 1-15 (cancelled).

16. (currently amended) A recombinant nucleic acid molecule encoding a modified pneumolysin polypeptide comprising one or more amino acid substitutions in a wild-type pneumolysin polypeptide having the amino acid sequence of SEQ ID NO:3, wherein said one amino acid substitution occurs at a position selected from the group consisting of position 61, 148, and 195, or wherein said more than one amino acid ~~substitution occurs~~ substitutions occur at positions selected from the group consisting of 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257, and wherein said modified pneumolysin polypeptide is soluble, elicits antibodies which are cross-reactive with wild-type pneumolysin, and has attenuated hemolytic activity.
17. (currently amended) A recombinant nucleic acid molecule comprising the pneumolysin nucleic acid sequence of SEQ ID NO: 1 or non-coding sequence changes thereof,
wherein said nucleic acid sequence comprises one or more of the nucleotide substitutions selected from the group consisting of:
A-50→G, G-54→T, T-181→C, A-196→T and T-302→C;
A-122→G, A-514→G, T-583→A and A-764→G;
A-187→T, T-380→A, A-382→C and T-443→A;
T-98→C, T-137→C, T-248→C, T-717→A and A-770→G;
T-134→C, A-305→G, A-566→G and T-583→G;
T-583→G;

T-583→A;

T-443→A;

and T-181→C.

18. (previously presented) The molecule of claim 16 or claim 17 as contained in a vector.
19. (currently amended) A genetically engineered microorganism or genetically engineered cell comprising the molecule of any of claims 16-17 or claims 32-40.
20. (currently amended) The genetically engineered microorganism or genetically engineered cell according to claim 19, wherein the genetically engineered microorganism or genetically engineered cell is selected from the group consisting [[of:]]of bacteria, yeast, mammalian and insect cells.
21. (currently amended) The genetically engineered microorganism according to claim 20, wherein the microorganism is *E. coli*.

Claims 22-26 (cancelled).

27. (withdrawn) A method for killing bacteria comprising contacting said bacteria with antibodies to an immunogenic molecule comprising a modified pneumolysin comprising one or more amino acid substitutions in a wild-type pneumolysin polypeptide having the

amino acid sequence of SEQ ID NO:3, wherein said one amino acid substitution occurs at a position selected from the group consisting of position 61, 148, and 195, or wherein said more than one amino acid substitution occurs at positions selected from the group consisting of 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257, and wherein said modified pneumolysin polypeptide is soluble, elicits antibodies which are cross-reactive with wild-type pneumolysin, and has attenuated hemolytic activity in the presence of complement.

28. (withdrawn) The method according to claim 27, wherein the immunogenic molecule is a polysaccharide-polypeptide conjugate wherein the polysaccharide is a bacterial capsular polysaccharide.
29. (withdrawn) A method for immunization of mammals comprising administering a vaccine comprising the modified pneumolysin polypeptide comprising one or more amino acid substitutions in a wild-type pneumolysin polypeptide having the amino acid sequence of SEQ ID NO:3, wherein said one amino acid substitution occurs at a position selected from the group consisting of position 61, 148, and 195, or wherein said more than one amino acid substitution occurs at positions selected from the group consisting of 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257, and wherein said modified pneumolysin polypeptide is soluble, elicits antibodies which are cross-reactive with wild-type pneumolysin, and has attenuated hemolytic activity and a pharmaceutically acceptable carrier to said mammals.

30. (withdrawn) A method for obtaining modified pneumolysin polypeptides, wherein said modified pneumolysin polypeptides have reduced hemolytic activity and are suitable for eliciting an immunogenetic response which is cross-reactive with wild-type pneumolysin comprising the steps of:

(a) mutating a nucleic acid molecule encoding wild-type pneumolysin to produce mutated nucleic acid molecules encoding modified pneumolysin polypeptides, wherein the modified pneumolysin polypeptides comprise one or more amino acid substitutions in a wild-type pneumolysin polypeptide having the amino acid sequence of SEQ ID NO:3, wherein said one amino acid substitution occurs at a position selected from the group consisting of position 61, 148, and 195, or wherein said more than one amino acid substitution occurs at positions selected from the group consisting of 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257 and expressing the mutated nucleic acid molecules in host cells;

(b) assaying the modified polypeptide expressed by the host cells for hemolytic activity; and

(c) identifying the modified pneumolysin polypeptides having substantially similar molecular weight as native wild-type pneumolysin and which are refoldable.

31. (currently amended) The molecule of claim 16 or claim 17, wherein the molecule is incorporated into a vector ~~[[is]]~~ selected from the group consisting ~~[[of:]]~~of a plasmid, cosmid, bacteriophage and yeast artificial chromosome.

32. (currently amended) A nucleic acid molecule encoding the nucleic acid sequence of modified pneumolysin polypeptide pNVJ1.
33. (currently amended) A nucleic acid molecule encoding the nucleic acid sequence of modified pneumolysin polypeptide pNVJ20.
34. (currently amended d) A nucleic acid molecule encoding the nucleic acid sequence of modified pneumolysin polypeptide pNVJ22.
35. (currently amended) A nucleic acid molecule encoding the nucleic acid sequence of modified pneumolysin polypeptide pNVJ45.
36. (currently amended) A nucleic acid molecule encoding the nucleic acid sequence of modified pneumolysin polypeptide pNVJ56.
37. (currently amended) A nucleic acid molecule encoding the nucleic acid sequence of modified pneumolysin polypeptide pNV103.
38. (currently amended) A nucleic acid molecule encoding the nucleic acid sequence of modified pneumolysin polypeptide pNV207.
39. (currently amended) A nucleic acid molecule encoding the nucleic acid sequence of modified pneumolysin polypeptide pNV111.

40. (currently amended) A nucleic acid molecule encoding the nucleic acid sequence of modified pneumolysin polypeptide pNV211.